

# EDICT OF GOVERNMENT

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PNS/BFAD 20 (2009) (English): Sugar Cane Wine (Basi) - Specification



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# PHILIPPINE NATIONAL STANDARD

PNS/BFAD 20:2009 ICS 67.160.10

Sugar cane wine (basi) - Specification



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#### Foreword

**Determination of Total Phenols** 

This Philippine National Standard Specification for Sugar cane wine (Basi) was prepared by the Technical Working Group under the Phase II Project for the Development of Standards for Selected Ethnic Food Products and was endorsed for adoption as Philippine National Standard by the Bureau of Food and Drugs.

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In the development of this standard different sugar cane wine samples were analyzed. Public consultation workshop was conducted in San Fernando, La Union where the product is commonly produced.

A recommended code of practice was developed simultaneously with this standard to serve as guide for the assurance of safety and quality and to make the locally produced sugar cane wines acceptable and globally competitive.

# PHILIPPINE NATIONAL STANDARD

# Sugar cane wine (Basi) - Specification

# 1 Scope

This Standard shall apply to alcoholic beverages prepared by fermentation of sugar cane *Saccharum officinarum* juice and/or its products.

# 2 References

The titles of the standards publications referred to in this standard are listed on the inside back cover.

# 3 Definitions of terms

For the purposes of this standard, the following terms shall mean:

# 3.1

# adjunct

it is a plant-derived product added to alcoholic beverages to contribute to their flavor and color

#### 3.2

# aging

it is the storing of wine in a sealed container after fermentation to improve its quality

# 3.3

#### brix

it is the concentration of sugar in syrup corresponding approximately to concentration of solutes expressed in percentage as measured with a refractometer or hydrometer and expressed in <sup>o</sup>Brix units

#### 3.4

#### container

it is any form of packaging material, which completely or partially encloses the food (including wrappers). A container may enclose the food as a single item or several units of types of pre-packaged food when such is present for sale to the consumer

#### 3.5

# contaminant

it is any biological or chemical agent, foreign matter, or other substances that are not intentionally added to food, which may compromise food safety and suitability

# 3.6

# current good manufacturing practices (cGMP)

it is a quality assurance system aimed at ensuring that products are consistently manufactured, packed or repacked or held to a quality appropriate for the intended use. It is thus concerned with both manufacturing and quality control procedures

10

# 3.7

#### ethanol

it is a light, volatile alcohol produced during fermentation of sugars

#### 3.8

#### fermentation

it is a metabolic process of converting reducing sugars into ethanol by yeast (Saccharomyces spp.)

#### 3.9

# food

it is any substance, whether semi-processed or raw, which is intended for human consumption, and includes drink, chewing gum and any substance which has been used in the manufacture, preparation or treatment of "food" but does not include cosmetics or tobacco or substances only used as drugs

#### 3.10

# food additive

it is any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including substance intended for use in the producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding of food; and including any source of radiation intended for any such use), if such substance is generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown scientific procedures to be safe under the conditions of intended use

# 3.11

#### food standard

it is a regulatory guideline that defines the identity of a given food product (i.e. its name and the ingredients used for its preparation) and specifies the minimum quality factors and when necessary, the required fill of the container. It may also include specific labeling requirements other than or in addition to the labeling requirements generally applicable to all pre-packaged foods

#### 3.12

# ingredient

it is any substance including food additive, used as a component in the manufacture or preparation of a food and present in the final product in its original or modified form

# 3.13

#### label

it includes any tag, brand, mark, pictorial or other descriptive script, written, printed, marked, embossed or impressed on, or attached to the container

# 3.14

#### lot

food produced during a period of time and under more or less the same manufacturing condition indicated by a specific code

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#### 3.15

pasteurization

it is a heat treatment process applied to a product with the aim of avoiding public health hazards arising from pathogenic microorganisms. Pasteurization, as a heat treatment process, is intended to result in only minimal chemical, physical and sensory changes

#### 3.16

packaging

it is the process of packing that is part of the production cycle applied to a bulk product to obtain a finished product. Any material, including printed material, employed in the packaging of a product including any outer packaging used for transportation of shipment. Packaging materials are referred to as primary or secondary according to whether or not they are intended to be in direct contact with the product

#### 3.17

# рН

it is the intensity or degree of acidity of a food material

# 3.18

phenols

these are weakly acidic organic compounds that contribute to the color, astringency and bitter flavor of wines

#### 3.19

processing aids

these are additives that are used in the processing of food to achieve a specific technological purpose and which may or may not result in the presence of residues or derivatives in the final product (BFAD A.O. No. 88-A s. 1984)

#### 3.20

potable water

it is the water fit for human consumption and potability determined by health authorities cited in Philippine National Standards for drinking water (Department of Health-Administrative Order No. 2007-0012: Philippine National Standards for Drinking Water 2007)

#### 3.21

#### refractometer

it is the instrument used to measure the percent soluble solids of sugars referred to as degree Brix (°Bx); concentration of sugars expressed in terms of number of grains of sucrose per 100g of liquid

# 3.22

titratable acidity

it is an amount of organic acids derived from the raw materials or produced during alcoholic fermentation, and expressed as grams of predominant acid per 100 mL of sample

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X

#### 3.23.

#### volatile acids

these are the steam-distillable acids present in the wine which is attributed to the growth of acetic acid bacteria and sometimes of yeasts; used as an indicator of spoilage and expressed as grams acetic acid per 100 mL of sample

#### 3.24

#### wine

it is an alcoholic beverage produced by the natural fermentation of the juice of grapes or other fruits or of the fermentable parts of plant or plant-related products; it contains 7 % to 24 % alcohol by volume and may contain certain optional ingredients

# 4 Description of products

#### 4.1 Product definition

Sugar cane wine, locally known as *basi*, prepared through the fermentation of sugar cane juice *or* its products, with or without the addition of optional ingredients like, but not limited to those stated in 5.1.2.

#### 4.2 Process definition

The product shall undergo a fermentation process followed by aging, may be pasteurized, and shall be filled in any suitable container sufficient to ensure quality and shelf life stability at ambient conditions.

# 5 Essential composition and quality factors

#### 5.1 Raw materials

# 5.1.1 Basic ingredients

- (a) Sugar cane juice Sugar cane juice to be fermented shall be prepared from a sound, clean and mature sugar cane variety, listed in, but not limited to Annex A, and/or from sugar cane products (such as refined and/or muscovado sugar) that is of a quality fit to be sold for human consumption.
- (b) **Inoculum** A starter culture essentially made up of yeast cells belonging to genus *Saccharomyces* and may include other fermenting microorganisms.
- (c) **Potable water** Water fit for human consumption.

# 5.1.2 Optional ingredients

Adjuncts – Usually take the form of dried plant materials, such as but not limited to samak bark, leaves and fruits, green guava (*Psidium guajava L.*) leaves, black plum/duhat (*Syszygium jambolanum*) bark, and ginger (*Zingiber officinale*).

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# 5.2 Quality criteria

# 5.2.1 General requirements

The sugar cane wine shall have the characteristic color, aroma and flavor of fermented sugar cane and should be free from objectionable sensory characteristics.

# (a) Alcohol content

The alcohol content shall compose mostly of ethyl alcohol and shall not be less than 12 % (v/v).

# (b) Methanol

The methanol content shall be in accordance to the provisions of BFAD M.C. No. 13 s 1989.

# (c) pH

The pH of the finished product shall not be less than 3.20.

# (d) Titratable acidity

The titratable acidity (as % lactic acid) shall not exceed 0.67 % (w/v).

# (e) Soluble solids

The soluble solids of the finished product shall not be less than 8.0 Bx.

# (f) Volatile acidity

The volatile acidity (as % acetic acid) shall not exceed 0.034 % (w/v).

#### (g) Total phenol

The phenol content (as mg gallic acid/mL) shall not be lower than 1.48 mg/mL

# 5.2.2 Types of defects

#### (a) Foreign matter

The presence in the sample unit of any matter, which has not been derived from sugar cane or from the processing aids used, does not pose a threat to human health and is readily recognized without magnification, or is present at a level determined by magnification method or any equivalent methods that indicates non-compliance with good manufacturing practices and sanitation practices.

#### (b) Odor/flavor/color

A sample unit affected by objectionable odors or flavors indicative of decomposition and unacceptable discoloration due to product deterioration.

# 5.2.3 Classification of "defectives"

A container that has any of the type of defects set in 5.2.2 shall be considered "defective".

# 5.2.4 Lot acceptance

A lot will be considered as meeting the applicable quality requirements when the number of "defectives", as defined in 5.2.3, does not exceed the acceptance number of the appropriate sampling plan.

#### 6 Food additives

Food additives when used shall be in accordance with the regulations established by the Bureau of Food and Drugs (BFAD) (Bureau Circular No. 016 s.2006. Updated List of Food Additives), the Codex Alimentarius Commission and/or authority for these products.

The following food additives listed in, but not limited to, Table 1, may be used for the manufacture of sugar cane wine (basi):

Table 1 – Food additives for sugar cane wine (*Basi*) (Codex Stan 192-1995. Codex General Standard for Food Additives).\*

Function	Additive	Maximum level
Color	Brilliant blue FCF	200 mg/kg
	Caramel III – Ammonia	GMP
	process	
	Caramel IV – Sulphite	GMP
	ammonia process	
	Carmines	200 mg/kg
	Carotenes, Beta-(Vegetable)	600 mg/kg
	Riboflavins	300 mg/kg
Preservative	Benzoates	1000 mg/kg (as benzoic acid)
	Dimethyl dicarbonate	250 mg/kg (added level; residue not detected in ready-to-eat food)
Antioxidant, bleaching agent	Sulphites	200 mg/kg (as residual SO <sub>2</sub> )
Emulsifier, sequestrant, stabilizer	Diacetyltartaric and fatty acid Esters of glycerol	5000 mg/kg
*Based on the food ca	tegory system: 14.2.4 Wines (Otl	her than grape).

#### 7 Contaminants

#### 7.1 Pesticide residues

Amount of residue shall comply with those maximum residue limits for pesticides established by the Fertilizer and Pesticide Authority of the Department of Agriculture, Codex Alimentarius Commission and/or authority for these products.

# 7.2 Heavy metal contaminants.

The products covered by the provisions of this standard shall comply with those maximum residue levels for heavy metal contaminants established by the Codex Alimentarius Commission and/or authority for these products.

# 8 Hygiene

- 8.1 It is recommended that the product covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of the Recommended International Code of Practice General Principles of Food Hygiene (CAC/RCP 1 1969, Rev. 4-2003) and/or the BFAD A.O. No. 153 s. 2004 Guidelines, Current Good Manufacturing Practices in Manufacturing, Packing, Repacking or Holding Food and processed according to the Recommended Code of Practice for the Processing and Handling of Sugar Cane Wine (Basi) (PNS/BFAD 21).
- **8.2** When tested by appropriate methods of sampling and examination, the product:
- shall be free from filth that may pose a hazard to health;
- shall be free from parasites which may represent a hazard to health;
- shall not contain any substance originating from microorganisms in amounts which may represent a hazard to health; and
- shall be free from container integrity defects which may compromise the hermetic seal.

# 9 Weights and measures

#### 9.1 Fill of container

# 9.1.1 Minimum fill

The minimum fill of container shall not be less than ¼ inch away from the cork or cap. A container that fails to meet the requirement for minimum fill shall be considered slack-filled.

#### 9.1.2 Lot acceptance

A lot will be considered as meeting the requirement of 9.1.1 when the number of slack-filled containers does not exceed the acceptance number of the appropriate sampling plan.

# 10 Labeling

- **10.1** Each container shall be labelled and marked with the following information in accordance with BFAD's labeling regulation (A.O. 88-B s. 1984):
- (a) The name of the product shall be "Sugar Cane Wine (basi)";

(b) The complete list of ingredients and food additives used in the preparation of the product in descending order of proportion;

(c) The net quantity of content by volume in the metric system. Other systems of measurement required by importing countries shall appear in parenthesis after the metric system unit;

(d) The name and address of the manufacturer, packer and/or distributor of the

food;

- (e) Lot or code number identifying product lot;
- (f) Open date marking; The words "Best/Consume Before"/"Use by date", may be included, indicating end of period at which the product shall retain its optimum quality attributes at defined storage conditions.
- (g) The words "Product of the Philippines" or the country of origin if imported;
- (h) Alcoholic Strength as a percentage by volume;
- (i) Directions for use;
  - Directions for use should be indicated on the label.
- (j) Storage instructions; and
- (k) Additional requirements.

A pictorial representation of the product and/or the raw material/s used should be placed on the label and not mislead the consumer with respect to the product and/or raw material so illustrated.

# 11 Methods of analysis and sampling

# 11.1 Measurement of pH of wines

According to the AOAC Official Methods of Analysis, Method No. 960.19, 16<sup>th</sup> ed., 1995. (Annex 2).

# 11.2 Determination of volatile acidity

According to the AOAC Official Methods of Analysis, Method No. 964.08, 16<sup>th</sup> ed, 1995. (Annex 3).

# 11.3 Determination of titratable acidity

According to the AOAC Official Methods of Analysis, Method No. 962.12, 16<sup>th</sup> ed., 1995. (Annex 4).

# 11.4 Determination of soluble solids

According to the AOAC Official Methods of Analysis, Method No. 932.14C, 16<sup>th</sup> ed., 1995. (Annex 5).

# 11.5 Determination of alcohol by volume from specific gravity

According to the AOAC Official Methods of Analysis, Method No. 920.57, 16<sup>th</sup> ed., 1995. (Annex 6).

# 11.6 Determination of total phenols

According to the AOAC Official Methods of Analysis, Method No. 952.03, 16<sup>th</sup> ed., 1995. (Annex 7).

# 11.7 Method of sampling

Sampling shall be in accordance with the FAO/WHO Codex Alimentarius Sampling Plans for Prepackaged Foods - CAC/RM 42-1969, Codex Alimentarius Volume 13, 1994.

# 11.8 Determination of lead using atomic absorption spectrophotometer

According to the AOAC Official Methods of Analysis, Method No. 972.15C, 16<sup>th</sup> ed., 1995.

# 11.9 Determination of tin using atomic absorption spectrophotometer

According to the AOAC Official Methods of Analysis, Method No. 985.16, 16<sup>th</sup> ed., 1995.

Annex A
Sugar cane varieties\*

Variety	Age of maturity (months)	Yield	Reaction to diseases
PHIL 56-226	10 to 12	High in tonnage and sugar content.	Resistant to leaf scorch, but susceptible to smut, downy mildew and yellow spot.
PHIL 58-260	12	High in tonnage and sugar content.	Resistant to leaf scorch, but susceptible to smut, downy mildew and yellow spot.
PHIL 62-120	10 to 12	High in tonnage with sugar content.	Highly resistant to downy mildew, resistant to smut, and leaf scorch.
PHIL 66-07	12	High in tonnage with sugar content.	Highly resistant to downy mildew, intermediate resistant to smut, and resistant to leaf scorch.
PHIL 67-23	12 to 14	Average in tonnage with high sugar content.	Resistant to smut and downy mildew, but intermediate susceptible to leaf scorch.
PHIL 72-28	12 to 14	Average in tonnage with high sugar content.	Very highly resistant to downy mildew, highly resistant to leaf scorch, but intermediate to smut.
PHIL 72-70	12 to 14	Average in tonnage with high sugar content.	Intermediate susceptible to smut and downy mildew, resistant to leaf scorch and yellow spot.
PHIL 74-64	12 to 14	Average in tonnage. High in sucrose content.	Resistant to smut, leaf scorch and mosaic virus.
PHIL 75-44	12 to 13	Average in tonnage, high in sugar content.	Highly resistant to smut, but susceptible to downy mildew.
PHIL 77-79	10 to 12	Average in tonnage, high in sugar content.	Resistant to downy mildew, average to leaf scorch and yellow spot, but susceptible to smut.
PHIL 80-13	10 to 12	High in tonnage and sugar content.	Resistant to smut, average to leaf scorch and susceptible yellow spot.
PHIL 80-93	12	Average in tonnage, high in sugar content.	Highly resistant to downy mildew and average to leaf scorch and yellow spot.
PHIL 83-61	12	Average in tonnage with average sugar content.	Resistant to smut, yellow spot and leaf scorch but moderately resistant to downy mildew.
PHIL 84-77	12	Average in tonnage with average sugar content.	Resistant to four major disease: smut, downy mildew, yellow spot and leaf scorch.
PHIL 85-83	10 to 12	High in tonnage and sugar content.	Resistant to smut, downy mildew, and leaf scorch but susceptible to yellow spot.

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Table 1 (concluded)

Variety	Age of maturity (months)	Yield	Reaction to diseases
PHIL 87-15	12	High in tonnage with average sugar content.	Resistant to downy mildew, leaf scorch but intermediate susceptible to smut.
PHIL 87-27	12	High in tonnage with average sugar content.	Resistant to smut, downy mildew, yellow spot.
PHIL 88-29	12	High in tonnage with average sugar content.	Resistant to smut, downy mildew and leaf scorch moderate resistant to yellow spot.
PHIL 88-35	12	High in tonnage with average sugar content.	Resistant to smut, downy mildew and leaf scorch, moderate resistant to yellow spot.
PHIL 88-39	12 to 14	High in tonnage and sugar content.	Resistant to smut and downy mildew.
PHIL 89-43	12	High in tonnage with average sugar content.	Resistant to smut, downy mildew, leaf scorch and susceptible to yellow spot.
PHIL 90-0345	12	High in tonnage and sugar content.	Resistant to smut, leaf scorch but susceptible to downy mildew.
PHIL 91-1091	12	High in tonnage and sugar content.	Resistant to smut, leaf scorch and to downy mildew.
PHIL 92-0051	12	Average in tonnage and sugar content.	Resistant to smut, leaf scorch, yellow spot and downy mildew.
PHIL 92-0577	12	Average in tonnage and sugar content.	Resistant to smut, downy mildew and leaf scorch moderate to yellow spot.
PHIL 92-0751	12	Average in tonnage and sugar content.	Resistant to smut, downy mildew, leaf scorch, yellow spot.
PHIL 93-2349	12	High in tonnage and sugar content.	Resistant to smut, downy mildew, and yellow spot; moderate to leaf scorch.
PHIL 93-3155	12	Average in tonnage and sugar content.	Resistant to smut and downy mildew; moderate to leaf scorch.
PHIL 93-3727	12	Average in tonnage and sugar content.	Resistant to smut and downy mildew; moderate to leaf scorch and yellow spot.
PHIL 93-3849	12	Average in tonnage and sugar content.	Resistant to smut and downy mildew; moderate to leaf scorch and yellow spot.

<sup>\*</sup>Other varieties of sugar cane not listed above may also be used provided that they conform to standards stated herein.

Reference:

Sugar Regulatory Adminsitration. www.sra.gov.ph

# Annex B

# Measurement of pH of wines

# B.1 Preparation of potassium hydrogen tartrate buffer solution (Saturated solution at 25 °C, 0.034 M)

Add excess (ca 100 %) of  $KHC_4H_4O_6$  (NIST SRM 188) to  $H_2O$  in glass-stoppered bottle or flask, and shake vigorously; few minutes of shaking is for saturation (100 mL  $H_2O$  at 25  $^{\circ}C$  dissolves ca. 0.7 g  $KHC_4H_4O_6$ ). Adjust to 25  $^{\circ}C$ , let solid settle, and decant clear solution, or filter if necessary. Discard when mold appears. Few crystals of thymol added during preparation will retard mold growth, and will alter pH by unit. For accuracy of  $\pm$  0.01 pH unit, temperature of solution must be between 20  $^{\circ}C$  and 30  $^{\circ}C$ .

# B.2 Calibration of pH meter

Let pH meter with glass and calomel electrodes warm up before use according to manufacturer's instructions. Check meter with freshly prepared, saturated, aqueous solution of  $KHC_4H_4O_6$ . Adjust meter to read 3.55 at 20 °C, 3.56 at 25 °C.

# B.3 Determination of pH of sample

Rinse electrodes free of bitartrate by dipping in H<sub>2</sub>O and then in sample. Place electrodes in fresh sample, determine temperature, and read pH to nearest 0.01 unit.

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#### Annex C

# Determination of volatile acidity

# C.1 Apparatus

- (a) Steam distillation apparatus See Figure 960.16 (see 26.1.32) of the AOAC Manual
- (b) Cash electric still See Figure 964.08 of the AOAC Manual. Consists of outer chamber, inner chamber, trap, 2-way stopcock, electric coil heater, and glass "T" outlet for  $H_2O$ . All parts are of Pyrex. Residue in inner chamber after distillation is flushed out automatically by vacuum action when current is shut off. Addition of  $H_2O$  through funnel above stopcock gives automatic spray bath to inner chamber, and waste drains through outlet in glass "T". Two-way stopcock permits introduction of sample, serves as escape vent for  $CO_2$ , and allows introduction of wash  $H_2O$ .

# C.2 Preparation of sample

Remove dissolved CO<sub>2</sub> from ca. 50 L sample by either: placing under low vacuum (H<sub>2</sub>O aspirator) 2 min with continuous stirring; or bringing to incipient boiling under air condenser and cooling immediately.

#### C.3 Determination

- (a) Steam distillation apparatus Add ca 600 L boiled H<sub>2</sub>O to outer chamber of still. Pipet 25 mL freshly prepared sample into inner chamber and stopper. Boil H<sub>2</sub>O 3 in with sidearm open. Close and distill ca 300 mL into Erlenmeyer. Add 0.5 mL phenolphthalein to distillate and titrate rapidly with 0.1 N NaOH until pink persists 15s. express results as g CH<sub>3</sub>COOH/100 mL = mL 0.1 N NaOH x 0.006 x 4.
- (b) Cash electric still Add H<sub>2</sub>O and pipet sample as in (a). Rinse funnel with ca 5 mL H<sub>2</sub>O. Distill ca 300 mL into Erlenmeyer. Titrate and express results as in (a). (Disconnect heating coil immediately and empty still by opening drain tube to outer chamber and stopcock to inner tube. Rinse still with two 10 mL 15 mL portions H<sub>2</sub>O by adding through funnel; evacuate each portion through drain tube.)

# Annex D

# Determination of titratable acidity in wines

Remove CO<sub>2</sub> if present, by either of the following methods:

- (1) Place ca 25 mL sample in a small Erlenmeyer flask and connect to H<sub>2</sub>O aspirator. Agitate 1 in under vacuum; or
- (2) Place ca 25 mL sample in a small Erlenmeyer flask, heat to incipient boiling and hold 30 s, swirl, and cool.

Add 1 mL phenolphthalein indicator solution to 200 mL hot, boiled  $H_2O$  in 500 mL wide-mouth Erlenmeyer flask. Neutralize to distinct pink. Add 5.00 mL degassed sample and titrate with 0.1 N standardized NaOH to same end point, using well-illuminated white background.

To express titratable acidity as grams of lactic acid per 100 mL of wine,

g lactic acid / 100 mL = mL NaOH x normality of NaOH x  $0.090 \times 100/5$ 

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# Annex E

# Determination of total soluble solids

# E.1 Apparatus

(a) Hand refractometer – With scale reading of 0 °Brix – 35 °Brix.

# E.2 Standardization of refractometer

Adjust instrument to read n of 1.3330 of 0 % sucrose with H<sub>2</sub>O at 20 °.

# E.3 Preparation of sample

Bring the sample to a temperature close to 20 °C, then filter to remove it of any undissolved solids

# E.4 Determination

Place sufficient amount of sample on the prism of the instrument, taking care that the sample covers the glass surface uniformly. Determine the total soluble solids by direct reading in terms of °Brix.

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#### Annex F

# Determination of alcohol by volume from specific gravity

# F.1 Distillation of sample

Measure 100 mL original material into 300 mL - 500 mL distillation flask, noting temperature, and add 50 mL water. Attach flask to vertical condenser by means of bent tube, distill almost 100 mL, and dilute to 100 mL at same temperature. (Foaming, which sometimes occurs, especially with young wines, may be prevented with by adding a small amount of antifoam material) For wines that contain an abnormal amount of CH<sub>3</sub>COOH, neutralize exactly with 1 N NaOH solution before proceeding with distillation (unnecessary for wines of normal taste and odor).

#### F.2 Calibration

Fill thoroughly cleaned pycnometer with recently distilled water, stopper, and immerse in constant temperature water bath with bath level above graduation mark on pycnometer. After 30 min, remove stopper and with capillary tube adjust until bottom of meniscus is tangent to graduation mark. With small roll of filter paper, dry inside neck of pycnometer, stopper, and immerse in water at room temperature for 15 min. Remove pycnometer, dry, let stand 15 min, and weigh. Empty pycnometer, rinse with acetone, and dry thoroughly in air with suction. Let empty flask come to room temperature, stopper, and weigh.

Weight of water = weight of pycnometer + water – weight of empty pycnometer

#### F.3 Determination of specific gravity at room temperature

1. Determine weight of sample as in F.2.

Weight of sample = weight of pycnometer + distillate - weight of empty pycnometer

2. Calculate specific gravity as follows:

Specific gravity = SW

where

S is the weight of sample;

W is the weight of water.

# F.4 Determination of alcohol

Obtain corresponding % alcohol by volume from Appendix C: Reference Volumes 913.02. AOAC Manual. 16<sup>th</sup> ed.

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#### Annex G

# **Determination of total phenols**

# G.1 Reagents

- 1. Folin-Denis Reagent To 750 mL water, add 100 g sodium tungstate (Na<sub>2</sub>WO<sub>4</sub>.H<sub>2</sub>O), 20 g phosphomolybdic acid (H<sub>3</sub>P(Mo<sub>3</sub>O<sub>10</sub>)<sub>4</sub>) x H<sub>2</sub>O and 50 mL phosphoric acid (H<sub>3</sub>PO<sub>4</sub>). Reflux for 2 hours, cool and dilute to 1 liter. Store in an amber bottle.
- 2. Sodium carbonate saturated solution To each 100 mL water, add 35 g anhydrous sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), dissolve at 70 °C 80 °C and let cool overnight. Seed supersaturated solution with crystal of Na<sub>2</sub>CO.10H<sub>2</sub>O and after crystallization, filter through glass wool.
- 3. Tannic acid standard solution 0.1 mg/mL. Dissolve 100 mg tannic acid in 1 L water. Prepare fresh solution for each determination. Gallic acid can also be used.

# G.2 Preparation of standard curve

Pipet 0 mL - 10 mL aliquots standard tannic acid solution into 100-mL volumetric flasks containing 75 mL water. Add 5 mL Folin-Denis reagent and 10 mL Na<sub>2</sub>CO<sub>3</sub> solution and dilute to volume with water. Mix well and determine the absorbance after 30 minutes at 760 nm. Plot absorbance against mg tannic acid/mL.

# G.3 Determination

Using 1 mL of sample, determine absorbance as in the preparation of the standard curve and obtain mg tannic acid/100 mL for the standard curve. If absorbance is too great (>0.7), repeat determination on 1 + 4 dilution of sample.



The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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#### FORMULATING BODY Development of Standards for Selected Ethnic Food Products - Phase II Standards for Sugar Can Wine. (Basi)

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